Cells on a Chip – the Use of Electric Properties for Highly Sensitive Monitoring of Blood-Derived Factors Involved in Angiotensin II Type 1 Receptor Signalling

Andrée Rothermel^{1*}, Randy Kurz^{1*}, Markus Rüffer¹, Winnie Weigel¹, Heinz-Georg Jahnke¹, Anna K. Sedello¹, Holger Stepan², Renaldo Faber², Kai Schulze-Forster³ and Andrea A. Robitzki¹

¹Center for Biotechnology and Biomedicine, Department of Molecular biological- biochemical Processing Technology, University of Leipzig, ²Department of Obstetrics and Gynecology, University of Leipzig, ³CellTrend GmbH, Im Biotechnologiepark Luckenwalde, *these authors contributed equally to this work

Key Words

Angiotensin receptors • Chronotropic agents • Cardiomyocytes • Renin angiotensin system • Biosensor • Preeclampsia

Abstract

Background: We developed a highly sensitive cardiomyocyte based screening system for the non-destructive electronic detection of chronotropic drugs and tissue-secreted factors involved in AT₁ receptor-mediated cardiovascular diseases. **Methods:** For this purpose we cultured spontaneously beating neonatal rat cardiomyocytes on microelectrode arrays (MEAs), and tested the optimised, stable culture parameters for a reproducible real-time recording of alterations in contraction frequency. After the evaluation of culture parameters, computer-based electronic measurement systems were used for counting of contractions by recording of the field potential of cardiomyocytes. **Results:** Using the biosensor, angiotensin II, the predominant ligand of the AT₁ receptor, was detected at very low concentrations of 10⁻¹¹ M via altered contractions of cardiomyocytes. Moreover, we demonstrated that cardiomyocyte coupled microarrays allow the detection of blood-derived low concentrated anti-AT₁ receptor

autoimmune antibodies of pregnant women suffering from preeclampsia. **Conclusion:** This study demonstrates the first well-suited electrophysiological recording of cardiomyocytes on multielectrode arrays as a benefit for functional biomonitoring for the detection of AT₁ receptor/ligand interactions and other marker proteins in sera directed to cardiovascular diseases.

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Introduction

The mammalian angiotensin II type 1 (AT₁) receptor is the predominant signal transducing component of the renin-angiotensin system [1]. Angiotensin II (Ang II), the preferred ligand of the AT₁ receptor is a key regulator of cardiovascular homeostasis, cardiac contractility and growth [2, 3]. Besides its physiological function, the pathological activation of the AT₁ receptor via Ang II plays a pivotal role in the development of a number of cardiovascular diseases such as cardiac hypertrophy, hypertension, myocarditis, cardiomyopathy, and myocardial infarction [4, 5]. Recently, it has been shown that autoimmune antibodies directed to the second

extracellular loop of the AT₁ receptor can also cause its activation and induce downstream signals leading to an increased contraction frequency [4, 6]. Interestingly, AT₁ receptor autoimmune antibodies (AT₁AA) are present in sera of pregnant patients suffering from preeclampsia or HELLP syndrome, and in transplanted patients with renal transplant rejection [6-11]. Assuming that binding of AT₁AA to the AT₁ receptors alters the contraction behaviour of rat cardiomyocytes, these cells can be used for the detection of low concentrated factors in serum samples by small alterations of contraction frequency. Since AT₁AA and autoimmune antibodies in general are present in extreme low concentrations in the blood an improved coupling of cardiomyocytes on multielectrode arrays possibly allows an enhancement of the detection limit. Unlike the technical component spontaneously beating neonatal rat cardiomyocytes are phenotypically and functionally unstable when cultured for longer periods [12], and therefore, they constitute the crucial component for the development of a highly sensitive biohybrid screening system. Another challenge when cultivating myocytes is that cardiomyocytes become overgrown by other cells such as fibroblasts after a few days in culture [13, 14]. It has been reported that this could negatively affect the contraction properties of cardiomyocytes and thereby influence bioelectrical recordings [15]. For simultaneous and automated real-time recordings of transmembrane voltage of cardiomyocyte cultures on 60 substrate-integrated microelectrodes, we determined (i) the influence of parameters such as cell adhesion and temperature on electrophysiological activity and contractility during cultivation, (ii) the time-dependent stability of the contraction rate for a reliable recording, (iii) the dose-response curve of Ang II to determine the sensitivity of the biosensor, we (iv) document the specific effect of Ang II via the AT₁ receptor antagonist losartan, and (v) proof in principle the bioelectronic monitoring of AT₁AA in sera of preeclamptic patients. According to the state of the art this report demonstrates for the first time, a highly sensitive functional screening system for real time monitoring of AT₁ receptor-mediated chronotropic and arrhythmic inducing factors.

Material and Methods

Isolation and cultivation of neonatal rat cardiomyocytes

For the isolation of neonatal rat cardiomyocytes, ventricular parts of the heart from one day old Sprague Dawley rats were minced into small pieces, collected in Hank's balanced salt solution (HBSS) and washed three times in HBSS. The

pooled heart pieces were incubated in an enzyme solution containing 0.2% trypsin for 15 min at 37°C and thereafter the supernatant was removed and discharged. After the addition of fresh trypsin solution to heart tissue and incubation for 15 min at 37°C, supernatant was collected and transferred in 50 ml tubes containing fetal calve serum (FCS). The digestion was repeated 3-5 times until tissue pieces were dispersed. The pooled cell suspension was centrifuged at 200 x g for 5 min at room temperature. The pellet was resuspended in SM20-I medium supplemented with 10% FCS, penicillin (100 U/ml), streptomycin (100 µg/ml), hydrocortisone (1 µg/ml) and CaCl₂ (15 µg/ml). SM20-I was developed for the CO₂-independent cultivation of cardiomyocytes (obtained from Biochrom AG). For the cultivation of cardiomyocytes, multielectrode arrays were coated with laminin (5 µg/ml in PBS). Thereafter, cells were plated at a density of 1000 cells/mm² and maintained in 1 ml SM20-I medium at 37°C in a humidified atmosphere. Culture medium was changed on alternate days and cardiomyocytes were monitored using an inverted microscope and by electronic recordings every day.

Immunocytochemical characterisation of cardiomyocytes

For immunocytochemical staining cardiomyocyte cultures were harvested at appropriate stages and fixed in 4% formaldehyde for 30 minutes at room temperature. After two washes in PBS, cardiomyocytes were stained with the monoclonal anti- α -actinin antibody (Sigma), and the polyclonal antibody anti-AT₁ receptor (Santa Cruz Technologies). Before application of the primary antibodies, fixed cardiomyocyte cultures were preincubated in a blocking solution (3% BSA, 0.1% Triton-X-100 in PBS) for 30 minutes at room temperature. Then, cardiomyocytes were incubated over night at 4°C with the appropriate primary antibodies at a dilution of 1:1000 (mouse anti- α -actinin 150 µg/ml, and rabbit anti-AT₁ receptor 200 µg/ml) followed by three washes in PBS. Subsequently, Cy3 conjugated secondary antibodies (1 mg/ml) were applied for 1 hour (1:100 in PBS, 0.1% Triton-X100). Between the last two washes, cell nuclei were stained with DAPI (0.1 mg/ml 4', 6-diamidino-2-phenylindol-dihydrochloride in PBS). Finally, cardiomyocytes were dried and embedded in Kaiser's glycerine gelatine. For documentation photomicrographs of stained cardiomyocytes were taken with a Nikon E600 or Nikon TE 2000 equipped with a monochromatic digital camera 1300, processed with LUCIA G, and Adobe Photoshop 7.0.

Semi-quantitative RT-PCR analysis of AT₁ receptor mRNA

For semi-quantitative RT-PCR total RNA was isolated from cardiomyocyte cultures of appropriate stages (Qiagen) and transcribed in cDNA by using the Reverse Transcription System from Promega. PCR was carried out as previously described [16]. The primers for the AT₁ receptor (sense primer: 5'-CCAATGGGGAGTGTGAGTT-3'; antisense primer: 5'-GGAAACAGCTTGGTGGTGAT-3') and GAPDH (sense primer: 5'-TCCTCAGTGTAGCCAGGAT-3'; antisense primer: 5'-ATGGGAAGCTGGTCATCAAC-3') were used to amplify cDNA fragments of 449 bp or 646 bp, respectively. After 30 cycles of

amplification, PCR fragments were separated by agarose gel electrophoresis and analysed by densitometric quantification (Biorad). The relative expression of the AT₁ receptor mRNA is shown as percentage of GAPDH band intensity for each culture stage. Accession numbers of AT₁ receptor: P25095; GAPDH (glyceraldehyde phosphate dehydrogenase): P04797.

Preparation of the Immunoglobulin G fraction from sera

To purify the IgG fractions from normal and preeclamptic pregnant women 1 ml serum was applied to an Affi-Gel Protein G column (Biorad) and performed according to manufacturers' instructions. The eluted IgG fraction was dialysed against PBS and concentrated by ultra-filtration using a centricon 20 (Millipore) with a membrane cut-off of 100 kDa. Prior to application, the protein concentration of the IgG containing eluate was determined according to Bradford [17].

Immunoblotting

Protein fractions (5 µg/lane) were diluted in 1x SDS loading buffer (without mercaptoethanol) and separated by SDS-PAGE on a 7.5% separation gel. For immunostaining proteins were transferred onto a polyvinylidene difluoride membrane (Carl Roth). PVDF membranes were preincubated in blocking buffer (5% non-fat dry milk, 10 mM Tris pH 7.5, 100 mM NaCl, 0.1% Tween 20) for 1 hour at room temperature. The membranes were rinsed three times in wash buffer (10 mM Tris pH 7.5, 100 mM NaCl, 0.1% Tween 20) and incubated for 2 hours with a horseradish peroxidase conjugated antibody against human IgGs (Dianova). Antibodies were visualised with diaminobenzidine and documented with a Gel Doc EQ System from Biorad.

Extracellular recording of cardiomyocytes using multielectrode arrays

For the extracellular recording of field potentials, cardiomyocytes were cultured on a planar multielectrode array with 60 substrate-integrated Titanium Nitride electrodes with a diameter of 10 µm and an interelectrode distance of 200 µm (Fig. 1). An internal electrode was used as reference electrode. To measure the electrogenic properties of cardiomyocytes the biochip was connected via a heatable MEA1060 amplifier system (Multi Channel Systems). Recording of electric activity of cardiomyocytes was carried out simultaneously on all electrodes at a sampling rate of 4 kHz. Data was analysed and processed off-line using a toolbox programmed with MATLAB (The Mathworks, Natick, MA, USA) to identify voltage signals [18, 19]. To analyse alterations in beating frequency the peak-to-peak distance of the negative amplitude of the field potential was calculated. For long-term experiments the same cardiomyocyte cultures were recorded starting from the first day after plating up to 7 days. The influence of temperature, Ang II and sera from normal and preeclamptic pregnant women on contraction frequency was analysed by using 5 days old cardiomyocytes *in vitro*. To determine the influence of temperature on contraction frequency, cardiomyocytes were gradually heated to the MEA1060 amplifier at a range from 27°C to 41°C. Temperature within the culture reservoir of the MEA was controlled by an external temperature sensor and correlated with data obtained by recording the beating

frequency. The efficiency of the cardiomyocyte based multielectrode array was determined by measuring the contraction frequency in response to various Ang II concentrations (10⁻⁶ to 10⁻¹² M). After determination of the basal beating rate, 10 µl PBS were added to cardiomyocytes *in vitro* to exclude unspecific effects due to the solvent of Ang II. Subsequently, Ang II was added, alterations in the beating rate were recorded and calculated off-line by using a software from MATLAB. For the detection of chronotropic effects of serum components derived from preeclamptic and non-preeclamptic pregnant women the purified IgG fractions were added in a final concentration of 10 µg/ml.

Results

Determination of optimal parameters for the cultivation and highly sensitive bioelectric recording of spontaneously beating cardiomyocytes

To determine the optimal parameters for cultivation and highly sensitive bioelectric recording, neonatal cardiomyocytes of one day old Sprague Dawley rats were isolated and cultured in CO₂ independent medium on microelectrode arrays (Fig. 1). For cultivation and recording of cardiomyocytes a culture reservoir on the top of the multielectrode array, which was hermetically sealed by a lid, allowed electric measurement under minimised environmental disturbance (Fig. 1A). Alterations in contraction frequency were recorded simultaneously on 60 microelectrodes online and in real time (Fig. 1B and C). At day 5 *in vitro*, cardiomyocytes showed a synchronised contraction as revealed by simultaneous onset of peaks on nearly all 60 electrodes (Fig. 1D). Long term recording of a single electrode demonstrated that the amplitude of the field potential increased over the time of cardiomyocyte culture (Fig. 1E). After 2 days in culture, voltage signals were recorded in a range of -100 µV to 50 µV. During further cultivation the positive and negative phase of the field potential was steadily elevated, reaching a peak maximum of 900 µV and a minimum of -1000 µV on day 5 *in vitro* (Fig. 1E). Thus, these intense field potentials allowed a reliable computer based automated peak detection.

Next, we analysed the tolerance of cardiomyocytes to temperature alterations at day 5 *in vitro*. For this purpose the temperature of the culture medium was gradually increased from 27°C to 41°C. As shown in Fig. 2A a linear correlation between the increasing temperature and contraction frequency of cardiomyocytes was detectable. For instance, a small shift in temperature of only 2°C, e. g. from 35°C to 37°C increased the contraction frequency by 10 contractions per minute (n=7,

Fig. 1. Cultivation and long-term recording of cardiomyocytes on multielectrode arrays (MEA). (A) The microelectrodes in the recording field are situated in the centre of the cell culture chamber which can be sealed with a lid for sterile cultivation and measurement. Via insulated interconnects and gold contact areas on the edge of the glass chip, the microelectrodes are connected to preamplifiers, analogous-to-digital converters, and to a computer. (B) Phase contrast image of neonatal rat cardiomyocytes cultured in a medium-containing chamber of a planar multielectrode array with 60 substrate integrated microelectrodes, each with a diameter of 10 μm and an interelectrode distance of 200 μm . Before plating of cells, multielectrode arrays were pre-coated with laminin to promote adhesion and growth of cardiomyocytes. (C) Magnification of (B) shows cardiomyocytes cultured for 5 days on a multielectrode array. At this stage, cardiomyocytes form a functional contracting monolayer that can be used for automated detection of contraction frequencies via recording of field potentials. (D) Computer based real time recording of field potentials from all 60 electrodes after 5 days *in vitro*. (E) Long-term recording of an individual electrode demonstrates the increase in peak intensity during cultivation.

Fig. 2. Contraction frequency in dependence on temperature and on duration of cardiomyocyte cultures. (A) At day 5, the temperature of the medium was increased from 27 $^{\circ}\text{C}$ to 41 $^{\circ}\text{C}$, controlled via a temperature probe and contraction frequency was recorded electronically. A temperature shift of only 2 $^{\circ}\text{C}$ increased contraction frequency by up to 10 contractions/minute ($n=7$, recording of 40-50 electrodes of 7 multielectrode arrays). (B) Long-term recordings showed that contraction frequency depends on the age of cultured cardiomyocytes. Electronic recording was carried out every day from at least 6 different multielectrode arrays ($n \geq 6$). From day 4 on contraction frequency was stabilised.

recording of 40-50 electrodes of 7 multielectrode arrays). This in turn demonstrated that temperature is a crucial parameter, especially for highly sensitive functional biomonitoring of small alterations in contraction frequency. Therefore, the following measurements were carried out on a heatable electronic board that was additionally placed in an incubator at 37 $^{\circ}\text{C}$.

Long term recordings of contraction frequency (Fig. 2B) revealed that cardiomyocytes reached a contraction frequency of 127 ± 22 ($n=12$; number of analysed multielectrode arrays) contractions per minute at day 2, 132 ± 12 ($n=9$) at day 3 and 91 ± 7 ($n=11$) at day 4. From day 4 on, contraction frequency of cardiomyocytes remained nearly stable at 97 ± 8 ($n=8$) contractions per

minute at day 5 and a contraction frequency of 87 ± 20 (n=8) at day 6. From day 8 on, areas of contracting cardiomyocytes were gradually reduced resulting in a decrease of electrodes that can be used for extracellular recordings (data not shown).

Molecular and immunocytochemical characterisation of neonatal rat cardiomyocytes

Besides a high field potential and a synchronised and stable contraction frequency, the existence of AT_1 receptors in cardiomyocyte cultures is a further essential parameter for highly sensitive recordings of AT_1 receptor mediated chronotropic effects. Therefore, the temporal expression pattern of the AT_1 receptor was analysed by semi-quantitative RT-PCR analysis (Fig. 3A and B). The time course of mRNA expression revealed a continuous increase from initially $2.7 \pm 0.5\%$ at day 2, to $2.9 \pm 0.8\%$ at day 4, to $6.1 \pm 1\%$ at day 6, and from $6.6 \pm 1.2\%$ at day 8 to $7.4 \pm 1.4\%$ at day 10 (Fig. 3B). Immunocytochemical stainings of 5 days old cardiomyocyte cultured on multielectrode arrays showed that AT_1 receptors were expressed over the entire surface of cardiomyocytes (Fig. 3C, left), whereas a more pronounced staining (red) occurred in small speckle-like structures in the peripheral margin of the cell bodies (cell nuclei stained blue). Furthermore, staining with α -actinin antibodies emphasised the cytoskeletal distribution of α -actinin in 5 days old cardiomyocyte cultures (Fig. 3C, right).

Electronic detection of extremely low concentrations of angiotensin II

Based on the findings mentioned above we decided to use 5 days old cardiomyocyte cultures for further multielectrode recordings. To demonstrate the efficiency of the cardiomyocyte-based biosensor for the reliable detection of chronotropic effects mediated by the AT_1 receptor, we first analysed the influence of the positive chronotropic peptide Ang II on the contraction rate of cardiomyocytes. Since Ang II was diluted in PBS, first of all 10 μ l PBS were applied to cardiomyocyte cultures to record and exclude any unspecific effects (Fig. 4A). The application of PBS (first line in Fig. 4A) did not increase contraction frequency, whereas Ang II in a final concentration of 10^{-8} M showed an elevation of the contraction frequency by 50% from 80 to 120 contractions per minute (second line in Fig. 4A).

Since Ang II can also activate the AT_2 receptor, which is rather expressed in embryonic tissues than in adult individuals [20], we used the AT_1 receptor antagonist losartan to determine whether the positive chronotropic

Fig. 3. Characterisation of cardiomyocytes grown on multielectrode arrays. (A) Semi-quantitative RT-PCR of the AT_1 receptor (left) and GAPDH mRNA (right) of cardiomyocyte cultures at different stages and subsequent agarose gel electrophoresis showed an gradual increase of AT_1 receptor expression. (B) Densitometric analysis of AT_1 receptor mRNA expression (% of GAPDH intensity). (C) 5 days old cardiomyocytes grown on multielectrode arrays were stained with an AT_1 receptor specific antibody (left) or with a heart muscle cell specific α -actinin antibody (right). Primary antibodies in (C) were visualized by Cy3 conjugated secondary antibodies (red), whereas nuclei were stained with DAPI.

effect of Ang II activates AT_1 but not AT_2 receptors [4]. Therefore, we treated 5 days old cardiomyocyte cultures for at least 5 minutes with losartan (final concentration 10^{-4} M, first line in Fig. 4B) before applying 10 μ l Ang II in a final concentration of 10^{-8} M (second line in Fig. 4B). The electronic recording showed that Ang II did not increase the contraction frequency of cardiomyocytes in the presence of losartan. The dose-response curve of Ang II (Fig. 4C) revealed that multielectrode array-coupled cardiomyocytes were able to detect positive chronotropic effects of Ang II in a very low concentration of 10^{-11} M.

Fig. 4. Response of neonatal rat cardiomyocytes to angiotensin II. To detect the expression of functional AT_1 receptors and to analyse the sensitivity of the biosensor, cardiomyocytes were cultured at 37°C for 5 days on multielectrode arrays. (A) To test the effect of the angiotensin II solvent, PBS was added after 5 min (first line). Application of 10^{-8} M angiotensin II (Ang II) to cardiomyocytes after 10.8 min (second line) led to an increase of beating frequency. (B) To analyse the effect of angiotensin II mediated by the AT_1 receptor, 10^{-4} M losartan (AT_1 receptor specific inhibitor) was added after 4.7 min (first line). The subsequent addition of 10^{-8} M angiotensin II (second line, 10.5 min) did not increase the beating frequency. Each dot in (A) and (B) represents the extracellular recording of a single field potential. (C) The dose-response curve of angiotensin II demonstrates the sensitivity of the biosensor ($n=9$, recording of 40-50 electrodes of 9 multielectrode arrays). Differences of contraction frequency before and after application of angiotensin II were shown. Even at an angiotensin II concentration of 10^{-11} M alterations in contraction frequency were detectable. Data in (A, B) were processed offline with the tool box programmed with MATLAB or with Microsoft Excel (C).

Electronic detection of AT_1 autoimmune antibodies in sera of preeclamptic women

Finally, the focus of this study was to demonstrate whether cardiomyocytes coupled to multielectrode arrays can be used for the detection of chronotropic effects of analytes such as autoimmune antibodies in serum of pregnant women suffering from preeclampsia. Due to the fact that these autoimmune antibodies of the IgG subtype bind to the AT_1 receptor and activate downstream signals leading to an increased contraction frequency, we tested partially purified IgG fractions of sera of preeclampsia positive pregnant women on cardiomyocyte-based multielectrode arrays (Fig. 5A). We found that IgG fractions of preeclamptic women caused a dramatic increase in contraction frequency when compared to sera of non-preeclamptic patients (Fig. 5B). The application of the purified IgG antibodies of preeclamptic women resulted in an increase of nearly 100 contractions per minute. Furthermore, the existence of IgGs in the purified and tested samples were investigated by western blot analysis. As shown Figure 5C, IgGs were detected in purified serum fractions from preeclamptic and non-preeclamptic women.

Discussion

In this study we established, optimised and standardised a cardiomyocyte based microelectrode array that allows the highly sensitive measurement of

chronotropic effects mediated by the AT_1 receptor. Although multielectrode arrays have been used previously [13, 15, 20], we demonstrated for the first time that this technique can be used to detect small quantities of biological active molecules in analytes of patients suffering from preeclampsia.

Since neonatal rat cardiomyocytes are phenotypically and functionally unstable when cultured for longer periods [14], they represent the crucial component for the development of a highly sensitive biohybrid screening system. Another problem of myocyte cell cultures is that cardiomyocytes become overgrown by other cells such as fibroblasts after a few days in culture [12]. It has been reported that this could negatively affect the contraction properties of cardiomyocytes and thereby influences bioelectrical long-term recordings.

The immunocytochemical characterisation of

Fig. 5. Effect of IgG fractions from preeclamptic and non-preeclamptic pregnant patients on contraction frequency of cardiomyocytes. Cardiomyocytes were cultured at 37°C for 5 days on MEAs in SM20-I Medium before online measurement. (A) After 10 min (line) 10 µg of a purified IgG fraction from preeclamptic serum was added to cardiomyocytes. An increase of contraction frequency from originally 140 contractions per minute to 210 contractions per minute was detected within the first hour after application of the IgG fraction. (B) Application of 10 µg of purified IgG fraction from normal pregnant women after 10 min (line) did not show any increase in contraction frequency. Data were processed offline with the tool box programmed with MATLAB. Each dot in (A) and (B) represents the recording of a single field potential. (C) Purified IgGs from preeclamptic and non-preeclamptic sera were subjected to electrophoresis on a 7.5 % SDS-PAGE and immunoblotted with a peroxidase-coupled antibody against human IgG. Both, partially purified preeclamptic and non-preeclamptic samples contained IgGs. The blot data are representative of three separate experiments.

cardiomyocytes revealed that almost all cells in culture represent cardiomyocytes and therefore, an excessive number of other cells e.g. fibroblasts can be excluded. Moreover, detection of highly organised α -actinin filaments reflecting a functional phenotype of adult cardiomyocytes and is consistent with the observed contractility in these cell cultures – and more importantly, we found that cells in culture express AT_1 receptor in a sufficient amount to detect its activation by AT_1 AA.

Long-term multielectrode recordings demonstrated that contraction frequency was stabilised after 5 days and remained constant up to day 7 in culture. Furthermore, the remarkable increase of field potential during cultivation and the elevated peak maximum might be based on an improved adherence of cardiomyocytes and their intensive contact with the surface of the chip electrodes. Moreover, we found that temperature is also an important and crucial parameter for the accurate recording since small variations in temperature lead to dramatic changes in basic beating frequency. The efficiency of the biosensor for the application as diagnostic and/or pharmaceutical screening tool was evaluated by the application of the chronotropic peptide Ang II. The dose-response curve of Ang II revealed that multielectrode array-coupled cardiomyocytes were able to detect positive chronotropic effects of Ang II in a very low concentration of 10^{-11} M. So far it was possible to detect alterations of contraction frequencies induced by Ang II only in a range of 10^{-8} M to 10^{-9} M by optical and manual counting of contractions [6, 8, 21]. In contrast to manual counting of contraction

frequency, comprising only extreme marginal counts per cardiomyocyte cultures, multielectrode arrays allow the real-time recording of contractions on 60 microelectrodes simultaneously. This in turn demonstrates the outstanding efficiency and sensitivity of the developed cardiomyocyte biosensor. Thus, functional biomonitoring [12, 22] carried out with living cells on a chip enables the detection of weak tissue secreted or blood derived factors via intracellular signal amplification that results in alterations of beating rates. Furthermore, in contrast to Ang II activation, which is characterised by a fast elevation of beating frequency by 35 beats per minute, the application of the IgG fraction of preeclamptic women results in an

increase of over 100 contractions per minute. Furthermore, the duration of the elevation comprises almost 2 to 3 hours whereas Ang II causes an elevation of the contraction frequency for only 30 to 60 minutes. Therefore, we hypothesised that this significant effect is possibly caused by different binding properties of Ang II and autoimmune antibodies to the AT₁ receptor.

In conclusion we demonstrate the first cardiomyocyte-based biosensor that allows highly sensitive online and real time recording of bioactive chronotropic components such as Ang II or AT₁AA in sera from preeclamptic, pregnant women. In almost the same manner other autoimmune antibodies, which are involved in cardiac dysfunctions [23] might be detectable and

therefore, this technique can be applied as a pre-diagnostic screening tool. Furthermore, the biosensor can also facilitate the detection of positive or negative chronotropic or arrhythmic effects elicited by novel drugs, toxic substances or various analytes.

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